

Data Supplements

Figure 1: Diagram of Retinal Dopaminergic Neurons

The body and dendrites of DA cells are red and their multiple axons black. The axonal endings of DA cells make GABAergic synapses (*asterisks*) with the cell body of AII amacrines (*white*). On the left and right is represented the circuitry of the OFF and ON components of the rod pathway. The AII amacrines receive their input from rod bipolars. They make glycinergic synapses with the axonal endings of OFF-cone bipolars (*left*) and gap junctions with the axonal endings of ON-cone bipolars (*right*). OFF and ON cone bipolars, in turn, synapse with the dendrites of OFF and ON ganglion cells. The *dashed line* marks the boundary between the OFF sublamina (strata 1 and 2) and the ON sublamina (strata 3-5) of the inner plexiform layer.

Figure 2: The Perikarya of DA Cells in the Intact Retina Receive GABAergic Synapses

A. Synaptic endings, intensely immunoreactive to an antibody to the synaptic vesicle protein synapsin 1 (*arrowheads*), are in contact with the surface of the perikaryon of a DA cell that is stained by an antibody to TH (*red*). **Inset:** the endings (*arrowheads*) that are immunoreactive for synapsin 1 (*green*) are also positive to an antibody to VGAT (*blue*)

and are therefore GABAergic. *IPL*, inner plexiform layer.

- B.** Synaptic endings, intensely immunoreactive to an antibody to VGAT (*arrowheads, blue*), are in contact with the surface of the perikaryon of a DA cell that is stained by an antibody to TH (*red*). **Inset:** the GABAergic endings (*blue, arrowheads*) are in register with two spots that are immunoreactive for the postsynaptic protein gephyrin (*green*). They are therefore presynaptic to the DA cell.

Figure 3: Synapses on DA Cell Perikarya do not Survive the Dissociation Procedure

The isolated perikaryon of a DA cell, identified by its TH immunoreactivity (*red*), contained cytoplasmic organelles stained with antibodies to synapsin 1 and 2 (*green*) and gephyrin (*blue*) and its surface was reconstructed in a Z series of confocal optical sections. During the dissociation and subsequent sedimentation of the cell suspension, two synapsin-positive synaptosomes, one large (*arrowheads*) and one small, adhered to the surface of the DA cell. No gephyrin is present at the site of contact between the large synaptosome with the DA cell (**Inset, lower right**), which rules out the presence of a GABAergic synapse. Note that organelles immunoreactive for synapsin and gephyrin are

present in the cytoplasm of the cell.

Figure 4: The Perikarya of DA Cells in the Intact Retina Contain VGAT-positive Organelles

The perikaryon of a DA cell in a retinal section is identified by the immunostaining of its cytoplasm with an antibody to TH (*red*). A small number of cytoplasmic organelles are immunoreactive for both synapsin 1 (*green*) and VGAT (*blue*).

Figure 5: Solitary DA Cells Possess Surface GABA_A Receptors

Optical sections from a confocal Z series of an isolated DA cell stained with an antibody to the $\gamma 2$ subunit of the GABA_A receptor (*green*) and an antibody to TH (*red*). The nucleus (*blue*) is counterstained with DAPI.

- A.** GABA_A receptors form clusters over the cell membrane and some may have been internalized. Clustering and endocytosis are due to crosslinking of the receptors by the anti- $\gamma 2$ antibody. In fact, living cells were incubated with the antibody before treatment with formaldehyde because the extracellular epitope of this antibody is denatured by fixation.

- B.** A grazing section through the cell periphery confirms that the clusters are on the cell

surface.

Figure 6: Probability of Occupancy of the GABA_A Receptor After Exocytosis

Left: Concentration-distance curves for a point source of 27,000 molecules in a plane.

Radius distance from the origin is shown in abscissa. Numbers over the curves are time points after exocytosis. The diffusion coefficient was set at $0.2 \mu\text{m}^2\text{ms}^{-1}$.

Right: The dose-response function $p(c)$ of GABA_A receptors is plotted against the radius distance.

The parameter c was obtained from the graph on the *left*.